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Extracellular vesicles: Unraveling their physiology and role in cancer

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Abstract
Extracellular vesicles (EVs) have been thoroughly studied from different physiological contexts and now there are strong evidences that they play an important role in cancer. They have a bimodal role in cancer wherein they can manipulate the local and systemic environment to aid in cancer growth and dissemination as well as program the immune system to elicit an anti-tumor response. The amount of information on their formation, nature of cargo contained within them and horizontal communication of different types of cancer EVs has expanded considerably in recent years. Herein, we discuss biogenesis, physiological functions and contents of exosomes as well as their contribution to tumorigenesis.

Keywords: Extracellular vesicle, Exosome, Cancer, Metastatic niche, biomarker

Introduction
A high level of coordination is required for the coexistence of many cell types within the same organism. This coordination is mediated by molecular mechanisms of intercellular communication and soluble factors have been considered the main players in this process [1, 2]. Signaling cascades in target cells is activated by these soluble factors which include secreted ligands that can bind plasma membrane receptors [3]. The important types of intercellular communication are: autocrine, in which the soluble factors act on the same cell which has released them; paracrine, in which the target cell is in close proximity with the secreting one; and endocrine, in which the secreted factors travel great distances through the blood to reach target cells [4]. Recently, a more complex and evolutionary conserved communication mechanism has emerged in which cells are now known to exchange information through the release of membrane bound particles known as extracellular vesicles [5]. Exosomes are nanovesicles released from many types of cells, but in relatively higher concentrations from cancer cells [6, 7]. The process of exosome production (known as Microvesiculation), results in the formation of small membrane-derived vesicles up to 100nm in diameter. These vesicles which are released into extracellular space by the process of exocytosis are of homogenous shape and density [8]. In the context of cancer, immune surveillance is a process by which precancerous and malignant cells can initiate an immune response that destroys transformed cells [9, 10]. In some of the cancers, an inflammatory microenvironment restricts the immune system from rejecting malignant cells, and thus promotes the development of tumors [11]. Multiple immune cell types, including T and B lymphocytes, macrophages, and natural killer (NK) cells regulate tumourigenesis [12, 13]. There are strong evidences that exosomes act as important mediators of extracellular signaling through the transfer of cellular material. Normal and malignant cell derived exosomes have now been recognized as important in tumourigenesis, apoptosis, and chemotherapeutic resistance. Exosomes contribute to tumorigenes from two complementary mechanisms. One is the modulation and restructuring of the cellular microenvironment to create the metastatic niche [14, 15], and second is the attenuation/modulation of tumor immune responses [16, 17]. In this context, exosomes from a range of cells induce micro environmental changes in tissue that facilitate tumor formation, at the same time disarming anti-tumor immune responses. This allows tumor cells to migrate, avoid immune detection, attach to secondary sites within the patient, and establish metastatic growth.

Formation and secretion

It is now clear that exosomes can be produced by most organisms (including bacteria) and can be identified in diverse ecosystem [18]. In humans, exosomes can be produced by all cell types [19]. Inside the cell, exosomes are initially produced by a process of invagination into endosomal membranes to create multivesicular bodies (MVBs) [20] which differentiate them from the shedding micro vesicle that forms from direct budding of the cell membrane [21]. A collection of proteins, termed as endosomal sorting complex required for transport (ESCRT), is required for the formation of exosomes and sorting of cargo into them [22, 23]. Finally the fusion of the MVB with the cell membrane results in secretion of exosomes. This fusion is dependent on several Rab GTPase proteins including RAB27A, RAB27B, RAB11 and RAB35 [24, 25]. Secretion can be inhibited experimentally by treatment with the ceramide biosynthesis inhibitor, GW4869 [26, 27].

Structure and Contents

Multiple families of proteins are present on the surface of exosomes which include tetraspanins (CD63, CD81, CD9), heat shock proteins (Hsc70), lysosomal proteins (Lamp2b) and fusion proteins (CD9, flotillin, Annexin) [28]. Out of these, the tetraspanins have received a lot of attention, because the triad of CD63, CD81 and CD9 has been widely used as exosome markers. However, the possible existence of single exosome-specific proteins is yet to be disclosed [29]. In contrast to other endogenously derived nanoparticle-like structures, such as shedding microvesicles and apoptotic bodies, most of the proteins described above are exclusively expressed on the exosome surface which enables them to be used as markers [30]. It is now clear that exosomes contain a wide variety of proteins, including membrane trafficking and cytoskeletal proteins, major histocompatibility complexes, signal transducers, heat-shock proteins, as well as many others [31]. More than 4,000 different proteins have been identified from purified exosomes [32]. This is particularly important as exosomes excreted from one cell are able to fuse with surrounding cells, and thus can initiate signaling responses [33] especially in tumourigenesis, as characteristics of the primary tumor are adopted by both surrounding and distant tissues. Many studies have now demonstrated the importance of exosomal proteins in cell function, especially to the role of these proteins in cancer. These proteins include the MET oncoprotein, mutant KRAS, and Tissue Factor, which are known to promote important processes in tumor formation, namely proliferation and coagulation [34-36].

Physiological functions

Exosomes have been known to perform a wide array of functions. Their role in intercellular communication, has received much attention as a basic characteristic, transporting RNA and proteins between cells [30, 37-40]. Transport of exosomes increased the expansion of the hematopoietic stem cell pool via uptake of embryonic stem cell-derived microvesicles, regenerated kidney epithelium and heart muscle tissue after ischemic injuries, and initiated coagulation due to their content of tissue factor [41-43]. Exosomes released from the neurons has been proposed as a possible way for sharing of pathophysiologically important components leading to neurodegeneration [44]. The release of exosomes by neurons could be modulated by synaptic activity indicating complexity of the role of these exosomes [44, 45]. The release of exosomes by immune and cancer cells emphasizes important aspects of these small extracellular vesicles in the human physiology [46, 47]. Exosomes are significant for the normal antigen presentation by dendritic cells, B and T cell activation and immune cell effector functions. Exosomes isolated from the serum of patients suffering from oral squamous cell carcinoma possessed similar surface protein markers as the parent cancer cell, e.g. they were highly enriched in Fas ligand. Incubation of these cancer cell derived exosomes and T-lymphoblasts from these patients induced apoptosis of the T-cells due to interaction with the Fas receptor [48]. Cancer cell-derived exosomes were also shown to induce expansion of regulatory T-cells *in vitro*, hence pointing towards another important factor in the immune escape of cancer cells [49]. Cancer cells share genetic components with each other via exosomes to increase their malignant potential, an exchange shown to be dependent on so called invadopodia and heparin sulfate proteoglycans [50-52]. These tumor cell-derived exosomes are able to improve the tumor niche, facilitating both tumor expansion and cancer cell metastasis [53-55]. Cancer cell-derived exosomes might also be beneficial as biomarkers for detecting cancer growth at early stages due to their change in characteristics compared to those secreted by non-malignant cells [55, 56].

Role in Cancer

A wide range of nucleic acids, especially exosomal microRNA or messenger RNA (mRNA) have been shown to be present in tumor-derived exosomes. It has been shown that exosomes containing these nucleic acids can transfer their cargo to the recipient cells and thus induce phenotypic changes [57, 58, 59]. Tumor-derived exosomes contain distinct microRNA profiles in many cancers, including prostate [60], lung [61], breast [62], and ovarian [6]. Several studies have also demonstrated the transfer of onco-microRNAs to target cells, with these microRNAs capable of modulating target pathways in host tissue [63-66].

The primary cause of cancer mortality is the metastatic spread of tumor cells from the primary site to other tissues within the body. This, however unfortunately remains the most poorly understood aspect of carcinogenesis. Normal cells within the microenvironment of the tumor are known to influence metastatic behavior, and there is evidence demonstrating that the successful formation of a metastatic deposit depends on the prior priming of the site for future metastatic growth [67]. A variety of cytokines and growth factors are released by primary tumor cells that firstly mobilize bone marrow derived cells and then recruit them to the site of future metastasis. This creates the suitable environment for incoming tumor cells, that is called the pre metastatic niche [15, 67]. In addition to the release of soluble mediators as individual molecules, tumor cells release exosomes containing complex mixtures of these molecules, including many known effectors of tumourigenesis [31]. Several studies suggest that exosomes educate bone-marrow-derived cells recruited to the pre-metastatic niche, to induce a phenotype that supports tumor cell metastasis.

By inducing apoptosis in cytotoxic T-cells and reducing proliferation of Natural Killer (NK) cells, exosomes have the ability to deactivate the cytotoxic component of the immune response [17, 68]. In response to Interleukin-2 mediated blocking by tumor-derived exosomes, NK cells were unable to be activated [69, 70]. Exosomes from melanosomes containing Fas ligand and tumor necrosis factor related apoptosis inducing ligand has also been shown to induce apoptosis of T cells [71, 72]. Through the down-regulation of the expression of T-cell activation signaling components, tumor-

derived exosomes prepared from the ascites of ovarian adenocarcinoma were found to have immunosuppressive properties [73]. Exosomes released by NK cells contain perforin and CD56, as well as granzyme B through which they can inhibit tumor growth [74, 75].

Exosomes represent bioavailable vehicles that are well tolerated, bioavailable, targetable to specific tissues, resistant to metabolic processes, and membrane-permeable. This makes exosomes ideal candidates for delivery of drugs, proteins, microRNA/silent interfering RNA (siRNA), and other molecules, that would otherwise be rapidly degraded. The potential therapeutic targeting of exosomes could take a number forms, some targeting, or modulating the intrinsic effect of exosomes in the prevention of tumorigenesis or metastasis, via interactions with tumor cells, stromal tissue, and immune cells. Other strategies aim to use exosomes to generate therapeutic effects, through their use as a vehicle for the delivery of anti-tumor agents, or priming of immune responses. The removal of exosomes from the circulatory system is an attractive therapeutic option in mitigating the metastatic effect of exosomes. Another therapeutic avenue involves the use of exosomes to efficiently deliver cargo such as drugs, microRNA's, and antigens to target recipient cells in order to treat tumorigenesis or metastasis. The potential use of exosomes to deliver targeted chemotherapeutics has been investigated in a number of studies [76, 77]. Exosomes may also provide an opportunity to deliver tissue-targeted siRNA and microRNA's to regulate gene expression within target cells [78, 79]. siRNA containing exosomes have also been shown to cross the blood brain barrier, targeting neuronal cells and knocking down their target protein by more than 60% with little toxicity [80].

Exosomes may provide an excellent biomarker to monitor the emergence, progression, and prognosis of cancer, as well as the efficacy of treatment regimes. Although few, studies have revealed exosomes can be readily detected in tumor tissue and many bodily fluids, and can be found in higher concentrations, both in tumor tissue, and the serum and plasma of cancer patients [81, 82].

Conclusion

The vast repertoire of proteins and nucleic acid that can be packaged within exosomes appears to reflect the extensive, diverse, and complex signaling potential of these nanovesicles. Scientists are now beginning to unravel the complex roles of exosomes, and although both *in vitro* and *in vivo* data clearly demonstrate the tumor modulating potential of exosomes, the extent to which these signaling pathways dictate tumorigenesis in patients is far from being fully understood. Exosome concentrations, though increased in cancer, are relatively tiny, and methods of exosome isolation tend to be time consuming and can be expensive while yielding samples that require further downstream purification. Perhaps the greatest challenge in the investigation of exosome function is understanding the balance between healthy and oncogenic exosomal signaling, the degree to which cancer exosomes corrupt or ablate healthy exosome signaling, and the extent to which these interactions dictate metastasis over the course of disease. What makes this particularly challenging is the complex and multifunctional nature of exosomal signaling. To fully appreciate the signaling potential of exosomes, studies will need to investigate the co-contribution of proteins, nucleic acids, and lipids to the observed phenotype. Dissecting and revealing the contributions of each exosomal component, and modifying this intricate signaling pathway to elicit the required therapeutic response, will undoubtedly prove the most significant challenge in the utilization of exosomes as biomarkers and drug targets.

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